THE RESPONSE OF THE AMPULLAE OF LORENZINI OF ELASMOBRANCHS TO ELECTRICAL STIMULATION

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INTRODUCTION

The biological function of the ampullae of Lorenzini of elasmobranch fish remains uncertain, although by now there is considerable evidence concerning the different types of stimuli capable of eliciting responses in the sensory nerves. The ampullae are sensitive to changes of temperature of less than 0.1 °C. (Sand, 1938), a sensitivity that would normally justify their description as thermoreceptors were it not for the anatomical specialization of the accessory structures of the sense organ, which cannot be accounted for in this way. Mechanical sensitivity has also been described (Murray, 1960), again adequate as a basis for sensory function, but the exact manner in which this sensitivity would be employed is unknown. Isolated preparations are sensitive to CO₂ (Hensel, 1957) but this is unlikely to be of importance to the fish, for the concentrations concerned are high (2–10% in the air surrounding the preparation).

In this paper a further type of sensitivity is described, namely, to electrical stimuli (and also, as a result, to salinity changes), and in particular to changes in the voltage gradient in the surrounding water. Behavioural responses of other fish to such electrical stimuli are known (e.g. Lissmann & Machin, 1958), but the sensory side of such responses has only been worked out in a few cases (Hagiwara, Kusano & Negishi, 1962).

MATERIAL AND METHODS

Various Raja species were used, mostly R. clavata, but also R. montagui, R. naevus and R. brachyura, and a few specimens of Scyliorhinus canicula. Nerve impulses were recorded with a conventional a.c. amplifier, C.R.O. and tape recorder, after a dissection similar to that described previously (Murray, 1960). Briefly, the nerve running from the mandibular group of ampullae was dissected clear for 2–3 cm., the branch from the lateral line was cut and the complexity of the recording was reduced by cutting the nerve into strands (Fig. 1). The sensitivity was checked as soon as the response of individual units could be detected, before the attempt was made to isolate a single unit. As far as possible the sense organ was left in situ, although the dissection had to be carried up to the base of the capsule to allow the lateral line branch of the nerve to be cut (Fig. 1, In). In situ preparations do not last as well as isolated ones, probably because of the accumulation of metabolites when the blood circulation stops, but good preparations maintain their sensitivity, and their resting discharge, for over an hour. For most of the experiments, especially with large fish, the head only was used, and except where so stated the whole preparation was submerged beneath sea water to a depth of 5–10 mm., the end of the nerve being lifted up out of the water.
on to a pair of Ag/AgCl wire electrodes. The final dissection to obtain sufficiently few active units was carried out at this stage.

Voltage gradients in the water were established from a 1-2 or 2 V. battery, through a variable series resistance, change-over switch and 20 x 5 mm. Ag/AgCl electrodes, which were mounted 5 cm. apart at the edge of the water-bath 20 cm. from the sense organs (Fig. 1, $S_1$). A calibration of the gradients near the sense organs was made using the a.c. amplifier set to a time-constant of 1 sec.

![Diagram illustrating the location of the mandibular group of ampullae in the ray, the extent of dissection necessary, and the methods of stimulation employed.](image)

In another series of experiments the head of the fish was left in air, and small currents were channelled along one of the jelly-filled tubes to the sense organ from a cotton-wick electrode placed on the opening; the circuit was completed through another Ag/AgCl electrode placed elsewhere on the skin (Fig. 1, $S_2$). Current strengths down to about $10^{-9}$ A. were measured directly with a Tinsley mirror galvanometer (sensitivity 300 mm./µA.), but below that they could only be calculated. Under such conditions where currents due to the junction potentials in the electrode circuit could not be ignored, a threshold measurement was only accepted as genuine if reversal of the battery polarity resulted in an equal but opposite effect on the resting impulse frequency.
RESULTS

When a voltage gradient is established or changed in the sea water overlying the ampullae of Lorenzini the impulse discharge frequency is altered. The frequency is most affected when the direction of the gradient is parallel to the jelly-filled tubes of the sense organ, being increased when the tube-opening is negative to the capsule, and decreased when the tube-opening is positive, with a reversed off-effect in each case. The response shows complete adaptation at low stimulus strengths, the frequency returning three-quarters of the way back to its initial value in under 5 sec., but with relatively strong stimulation the adaptation is neither so complete nor so rapid (Fig. 2).

![Figure 2](image-url)

Fig. 2. The response of a single unit to voltage gradients in the water. Lower graph, impulse frequency; upper graph, stimulus. Strength of stimulus = 64 μV./cm.

Since in theory there is no threshold when a continuously variable stimulus is applied to a sense organ with a resting discharge, an arbitrary figure was used, namely, 10% change in impulse frequency lasting for a second. Such a small response is a possible criterion of threshold because the resting discharge is usually so regular, and in practice represented what could be heard on the loudspeaker when the unchanged activity of 1 or 2 other units was being recorded at the same time; if 3 to 4 other units were active, the audible threshold was somewhat higher. Fig. 3A shows the 67 most sensitive units found, and this includes 8 from Scyliorhinus, in which the thresholds are similar. These experiments are unrealistic in that the base of the capsule had been uncovered by the dissection and the nerve was led up through the sea water to the recording electrodes, making it possible for the electrical stimulation to act directly on the nerve. However, comparable sensitivities were recorded when the nerve and the base of the capsule were surrounded and so insulated by a plastic cylinder of 10 mm. diameter filled with liquid paraffin. Stimulation via the nerve rather than via the jelly-filled tube is also unlikely, because in experiments described later with the preparation in air, the threshold for stimulation via the tube was much lower than for stimulation of the ampulla via the nerve.
The direction of the gradient in the water which is most effective lies parallel to the tube, as mentioned earlier, and this holds even when the tube bends back at an acute angle away from the general direction of the majority of the tubes. Thus for an ampulla in the mandibular capsule the polarity which is normally excitatory is with the centre-line of the fish negative to the side on which the preparation has been made. The unit recorded in Fig. 2, however, appeared at first to be anomalous, for the polarity to excite was reversed. But on further investigation the tube-opening was found to lie slightly antero-lateral to the capsule (Fig. 1, o2), and therefore this preparation confirms the general conclusion. Thus it is the spatial relationship of tube-opening to capsule which is important, and not the nerve-capsule relationship, and this confirms that the effect of the voltage gradient on the nerve endings is via the tubes and not via the nerve itself.

Thus it appears that it is the current flowing along the jelly-filled tubes which is important in stimulating the nerve endings; this was tested directly, with the head of the fish in air, and the stimulating current was applied through an electrode resting lightly on the opening of one of the tubes (i.e. the tube corresponding to that sensory unit whose activity was recorded). The other electrode could be placed anywhere else on the preparation, and in practice all the threshold experiments were performed with it placed contralaterally (Fig. 1, S2). The resting discharge is speeded when the electrode on the end of the tube is cathodal, with an inhibitory after-effect, and is slowed with the tube-end anodal, with a post-inhibitory rebound. The time course of adaptation is similar to that described above. The threshold current strengths for the twelve most sensitive units are shown in Fig. 3 B. If the electrode is displaced from the tube opening, for example by 0.5 mm., the current is far less effective. In these experiments the skin surface remained moist, because of its mucous covering.
Pulsed electrical stimulation is also effective. Preliminary experiments in which the stimuli were applied through the water show that a train of short pulses produces the same pattern of spike discharge as does d.c. stimulation of equivalent total charge (Fig. 4); in this experiment the pulse:space ratio was 1:200, and the pulse voltage divided by 200 equalled 30 μV./cm. gradient in the water, which is near the d.c. stimulus required to produce a similar change of spike frequency. During pulsed stimulation the pattern of spike discharge is no longer regular, and there is a tendency for the spikes to occur more frequently just after an excitatory pulse and less frequently just after an inhibitory one. It is therefore technically easier to assess the effectiveness of a stimulus by observing the post-inhibitory rebound, for example (Fig. 5), after 1 sec. stimulation at constant intensity but variable duration and repetition frequency. It can be seen from the figure that doubling the frequency approximately compensates for halving the duration of the individual pulses. More exact quantitative experiments using pulsed stimulation are in progress.

In a separate series of experiments in which the head of the fish was submerged, fine, but slow, jets of slightly diluted or concentrated sea water were aimed at the opening of the tube whose nerve was on the electrodes. With each preparation a control was made with sea water to check that mechanical stimulation was not occurring; the opposite effects of dilution and concentration afford a separate control for this. All solutions were kept at the same temperature. The concentrations of the test solutions are expressed as percentages of normal sea water (which had a salinity of about 33%). Diluted sea water causes an increase in the resting frequency, with a corresponding reduction on return to sea water; concentrated sea water causes slowing. The time course of adaptation is the same as when d.c. stimuli are used, allowing for the slower onset of the stimulus (i.e. the sea water just at the entrance to the tube does not immediately get washed away) which results in a rounding-off of the peak of the
response. The effect was the same whether the dilution of the sea water was made with distilled water or with isotonic sucrose solution. Fig. 3C illustrates the threshold sensitivity of 17 units, and Fig. 6 shows the stimulus–response relationship for one of them. Since it had a resting discharge of 30–40 impulses per second, the change of frequency adopted here as the indication of threshold was 3–4 impulses per second,

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Fig. 5. The post-inhibitory rebounds in a single unit occurring after stimulation for 1 sec. with pulsed stimuli of constant amplitude but variable duration and frequency. The stimulation resulted in complete inhibition in each instance. Each record is the mean of 2–4 repetitions.

and from Fig. 6 it can be seen that this change of frequency was given in response to changes of concentration of 2.5%. This particular preparation responded to a voltage gradient of 1.5 μV./cm., could be inhibited by touch with a hair on the tube opening and had the characteristic ‘paradoxical’ sensitivity to temperature changes in the water touching the capsule.

The lateral line organs, which lie just posterior to the mandibular ampullae, are not nearly so sensitive to voltage gradients, and the threshold for one preparation was 10 mV./cm. The lateral line organs are in these fish situated in a thick-walled canal relatively deep in the dermis.
Response of elasmobranchs to electrical stimulation

Fig. 6. The response of a single unit to changes in the salinity of the sea water at the tube opening. •, mean frequency over the first 3 sec. after application of the test solution; ■, mean frequency over the first 3 sec. after the return to sea water. The results are plotted in terms of the change of impulse frequency from its initial level, as the latter varied between 30 and 40/sec. during the course of the experiment. Dilution of sea water was made with isotonic sucrose, and each test solution was applied for 15 sec.

DISCUSSION

Voltage gradients in the water over the ampullae will result in current flow along the jelly-filled tubes. Since the diameter (say 0.4 mm. for these ampullae) and the conductivity (0.04 mhos, Murray & Potts, 1961) are known, it is possible to calculate the current on the assumption of a tube open to the surface at both ends. The threshold current for a gradient of 1 μV./cm. would then be $5 \times 10^{-11}$ A. This is of course the upper limit, because no allowance has been made for the high resistance of the ampulla, capsule, tissues and skin that lie at the closed end of the tube. However, this figure of $5 \times 10^{-11}$ A. may be compared with the $4 \times 10^{-10}$ A. found by direct experiment with the electrode on the end of the tube, in air. The difference by a factor of ten times may probably be explained by the fact that the electrode was large compared with the size of the tube-opening, and much of the current could have leaked directly to the other electrode along the surface of the moist skin, so that only a tenth part or less of the total current was actually routed along the tube to affect the ampulla.

There is a further figure for current which can be compared with these two; in experiments in which a polarizing current was applied to the preparation via the sensory nerve itself the equivalent threshold current was $5 \times 10^{-8}$ A. with the electrode on the base of the undivided nerve, or $5 \times 10^{-9}$ A. when it was on the fine strand from which the single unit record was made (Murray, 1959, and unpublished). It seems reasonable when considering the current per tube to make an allowance for the number of sensory nerve fibres among which this total current was distributed, and figures of 100 and 10 fibres probably correspond to the two values of the threshold current.
The current per ampulla would therefore be approximately $5 \times 10^{-10}$ A. This is also an upper limit, because not all the current will have entered the fibres, and of that part which has so entered under the electrode, only part will remain inside up to the ending where the impulses are initiated. The comparison of the three methods of stimulation is relevant to the question whether the nerve endings are stimulated directly by the current flowing down the tube, or whether the secondary sensory cells are interposed as an amplifying stage, possibly with chemical transmission to the nerve endings. The similarity of threshold reduces the necessity for the latter hypothesis.

The sensitivity of fish to electrical stimuli so far described in the literature falls into one of two categories, with no overlap. There is the navigational function in fish with weak electric organs (Lissmann, 1958), and there are the usually quite unnatural conditions of galvanotaxis. From behavioural experiments on Gymnarchus niloticus Lissmann and Machin (1958) calculate that the fish must be sensitive to changes in voltage gradient in the water of 0-03 $\mu$V./cm. (a more reliable figure is 0-15 $\mu$V./cm., Machin & Lissmann, 1960), and in the other main group of weak electric fish Hagiwara, Kusano & Negishi (1962) have recorded electrophysiologically in Gymnotus and Hypopomus a sensitivity to differences in voltage gradient of 50 to 1000 $\mu$V./cm. On the other hand, the voltages involved in galvanotaxis are so great that the response seems to be a genuinely ‘forced’ movement due to differences of muscle contraction on the two sides of the body, neither the lateral line nor the cutaneous receptors being involved (Spiecker, 1957); moreover, the stimuli are often so strong that electronarcosis occurs (Miyake & Steiger, 1957). Actual values for the voltage gradients include 60 to 200 mV./cm. for 6 msec. pulses at 10 per sec. (Miyake & Steiger, 1957) and 10 to 100 mV./cm. (quoted by Lissmann & Machin, 1958). The ampullae lie therefore within the range of sensitivity found in fish which use their electric organs for navigational purposes.

Now it is true that rays have an electric organ in the tail which can produce pulses of 4 V. lasting for 0-5 to 1 sec. (see Fessard, 1958); and this would be a stimulus about 1000 times greater than threshold according to the sensitivity here described. But there is no evidence of rays using their electric organs for purposes of navigation, and in any case this can be no explanation of the function of the ampullae in dogfish.

So the general function of the electrical sensitivity of the ampullae is unlikely to be the reception of the individual’s own electric organ discharge; and the detection of the discharge from other electric fishes would also seem too restricted a function. Among the other possibilities are the detection of salinity changes, which will be discussed later, and also of the weak currents which result from the movement of conductors through the earth’s magnetic field. This last possibility can be briefly mentioned, although it would appear to be about one order of magnitude too small for the ampullae to detect under normal conditions. When either a fish or the sea water itself moves through the earth’s magnetic field, a potential gradient will be established in it. The potential gradient, the direction of movement and the magnetic field are all at right angles to one another, and if this effect is to be used for navigational purposes, it is the horizontal not the vertical component of the earth’s field which will be important. Thus for east–west movement of the fish, a vertical potential gradient will be established in it of 0-1 $\mu$V./cm./knot speed, and the polarity of the gradient will be different for easterly and for westerly movement (the currents flow towards dorsal and ventral
The response of elasmobranchs to electrical stimulation surfaces, respectively). The voltages will most readily be detected when there is relative movement between the fish and the water, otherwise the voltages induced in both will be equal, and there will be no local current flow around the fish. The other difficulty arises from the adaptation of the electrical response, so that only accelerations and not constant velocities are effective. However, although Gymnarchus could probably tell which way it was heading, it is unlikely that elasmobranchs can.

The sensitivity of the ampullae to changes of about 3-5% in the salinity of the surrounding water is certainly adequate for changes of impulse frequency to occur in the natural conditions of life. Rays and dogfish are found far enough inshore off estuaries for changes in salinity greater than this to be encountered, and there is often so little mixing that the boundary between the two water masses is quite sharp. But it is not certain that salinity detection is the main function of the ampullae, and it may just be a necessary corollary of the high electrical sensitivity; for the diffusion potentials which are found between saline solutions a few per cent different in concentration are relatively large compared to the sensitivity of the ampullae. Even though the actual situation is more nearly skin—normal sea water—dilute sea water—ampulla jelly—tissues, so that any junction effect between the jelly and the dilute sea water is balanced by the opposite effect between the dilute and the normal sea water, the differences in composition of jelly and sea water (Murray & Potts, 1961) may be enough to make the situation sufficiently asymmetrical. There is another possibility, namely, that the dilution of the sea water at the end of the tube alters a Donnan equilibrium potential which might result from the presence of fixed anions in the jelly. The macromolecular structure of the jelly is being investigated with these possibilities in mind.

In general, the ampullae afford an example of a sense organ in which the doctrine of the peripheral analysis of the modality of sensation breaks down. For whichever of the physiologically effective stimuli is in fact the biologically adequate one, there can be little doubt that nerve impulse traffic will be affected by all such stimuli, under natural conditions. So there must be some mechanism which will enable the central nervous system to distinguish between the various modalities of stimulation and react appropriately to them. The patterning in space and time of the impulse discharge from all the receptors must presumably be important; and, for example, temperature changes will affect most rapidly and most completely those ampullae which lie superficially close to the gill chambers, whereas mechanical and electrical stimuli will affect similarly those ampullae whose tubes open adjacent to one another on the surface. Comparison with responses in the lateral line nerves must also be useful.

A final point concerns the polarity of the stimulus. Increase in impulse frequency occurs when the cathode is above the sensory epithelium, and the anode below. This is exactly opposite to the situation in the lateral line organs of teleosts (Katsuki & Yoshino, 1952) and of *Xenopus* (Murray, 1956) and in the mammalian cochlea (Tasaki & Fernandez, 1952) where it is the anode above the epithelium which is excitatory. The explanation of the difference may be found in the difference of anatomical detail of the relation of stem axon and non-myelinated terminals to the path of the current. In the ampullae the end of the myelination lies close up against the base of the epithelium lining the ampulla itself, and so is readily accessible to the stimulating current, whereas in the lateral line organs the myelinated part of the nerve lies deep, away from the epithelium. In each case, therefore, the excitatory
current could result in depolarization at the site of impulse initiation (see Murray, 1956). If, however, in the ampullae the secondary sensory cells act as an intermediate stage in the transduction of the electrical stimuli into nerve impulses, there is no reason why the polarity for stimulation should be the same as in the lateral line organs.

SUMMARY

1. The ampullae of Lorenzini are sensitive to weak electrical stimuli which presumably cause currents to flow along the jelly-filled tubes of the sense organ. Increase of the resting frequency occurs when the tube-opening is made negative to the capsule, and inhibition when the opening is positive, with opposite after-effects in each case. Adaptation is three-quarters complete in about 5 sec.

2. When the stimulus is applied as a voltage gradient in the water overlying the ampullae, the threshold for the most sensitive units is 1 μV./cm., which represents a current along the tube of less than $5 \times 10^{-11}$ A.

3. When the stimulus is applied as a current directly to the tube opening, the threshold for the most sensitive units is $4 \times 10^{-10}$ A.

4. Changes in the salinity of the water at the tube opening are also effective, the threshold being about 3%. Dilution causes excitation, and concentration causes inhibition. The response is not due to the osmotic differences, and so probably results from potentials established at the jelly–water interface.

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REFERENCES


